122804

DATA EVALUATION RECORD

1. CHEMICAL: Avermectin B_1 (MK-936).

2. FORMULATION: Technical, 90.5%.

3. CITATION: Ward, G.S. 1983. Acute toxicity of MK-936 Technical to embryo-larvae of eastern oysters (Crassostrea virginica). Prepared by EG&G Bionomics, Pensacola, Fla.; submitted by Merck Sharp & Dohme, Three Bridges, N.J.. Reg. No. 50658-EUP-R. Acc. No. 252115

4. REVIEWED BY: John J. Bascietto
Wildlife Biologist
EEB/HED

5. DATE REVIEWED: 3/16/84

6. TEST TYPE: Acute toxicity estuarine/marine - 48hr EC_{50}

A) eastern oyster larvae (Crassostrea virginica)

7. REPORTED RESULTS:

48-hour EC_{50} = 430 ug/l (280-580) ug/l.
There is a statistically significant reduction of normal embryo development at >400 ppb.

8. REVIEWER'S CONCLUSIONS: The study was performed in a scientifically sound manner but the author's conclusions are based on unsupported assumptions regarding dose-related effects. A statistically significant reduction in the number of normal larvae was observed at as low as 50 ppb, but the author discounted the significance of this observation. ~~The required raw data on replicates was not submitted. The study does not fulfill the guidelines requirement for marine/estuarine acute toxicity with oysters at this time, but may be repaired by submission of the raw data on the replicates and reevaluation of the "statistical significance" of the effects observed.~~

The RAW DATA on replicates was submitted accepted 3/26/85. Upgrade to Core - John Bascietto

9. Materials/Methods

A. Protocol

The study used technical grade Avermectin (MK-936). A protocol for a static, unaerated 48-hr duration was used to study the reduction of "normal" 48-hr (veliger stage) larvae as compared to a nanograde acetone solvent control.

Natural, unfiltered sea water the initial medium. Embryos were obtained by induction of spawning by mature adults maintained on flowing unfiltered seawater at the testing facility. Adults were maintained for "several" months at the lab.

Seawater used in the test chambers was filtered through 5 um pores size polypropylene core filter. All concentrations were tested in triplicate in 0.95-1 glass jars. The toxicant was added by using an appropriate volume of stock solution (9,000mg/l - 0.9948 g of material + 100 ml of acetone). The solvent control contained the greatest volume of acetone added to test vessels (320 ul). A seawater control was also run.

Each vessel was inoculated with an estimated 25,878 embryos within 1 hr. of fertilization, and maintained at 21 + 1°C under ambient lighting conditions. After induced spawning and fertilization with oyster sperm (successful fertilization estimated at $\geq$ 90%) the embryo density was determined by a Sedgewick-Rafter count of a 1:10 dilution).

At 48-hr of exposure to 50,100, 200, 400, 800, 1600 and 3200 ug/l (nominal) the larvae were rinsed into a glass tube, through a 37 um mesh sieve, with 24 ml of filtered test seawater. The were preserved with formalin (1 ml each container). A Sedgewick-Rafter count was used to determine the number of "normally developed" larvae at 48-hr. (veliger stage - full shelled, straight - hinged).

B. Statistical Analysis

Percentage of reduction of "normals" was calculated by

$$\text{Percentage reduction} = \frac{\#\text{ normal solvent control} - \#\text{ normal in each test conc.}}{\#\text{ normal solvent control}} \times 100$$

This was the criterion for effect and is expressed as a 48-hr EC_{50} which was calculated by the Stephan, 1977 computer program for LC_{50}. This EC_{50} was determined by the moving average angle method of that program.

The number of normal larvae in all treatments was compared to those in the solvent control by ANOVA with William's method of multiple comparisons, to identify statistical significance ($P \leq .05$).

10. Results

(Only mean ($\bar{x}$) numbers of larvae from 3 replicates were reported).

Conc. (Nominal)	#Normal larvae $\bar{X}$	S.D.(a)	% Reduction of Normal larval
Control	23,718	1,788	-
Solvent Control	24,162	1,384	-
50 ppb	21,058	975	13[b]
100 "	22,357	2,460	7
200 "	21,217	898	12[b]
400 "	22,167	428	8
800 "	2,153	2,491	91
1600 "	19	33	100
3200 "	0	-	100

the author states "because of the lack of significance in 100 and 400 ppb and the variation in numbers of larvae between replicate test containers, we do not believe the significance "...(observed at 50 and 200 ppb)... "to be correlated to the toxicity of the test material".

(a) standard deviation

(b) Significant ($P \leq 0.05$) reduction in number of normal larvae as compared to solvent control (by ANOVA with William's method of multiple comparisons).

11. Reviewer's Evaluation

A. Protocols - the procedures followed were acceptable for acute toxicology with oysters under current guidelines.

B. Statistics - EEB did not validate the statistical analysis because the raw data was not submitted. We note two problems however. At this time we do not agree with the author's discounting of the statistical significance observed at 50 and 200 ug/l (see "Results" above). We also note that large and small standard deviations from mean number of normal larvae necessitate review of the raw data on the replicates.

C. Results - A review of the mean number of normal larvae as compared to the acetone control suggests that the EC_{50} is between 400 and 800 ppb. But large S.D.'s necessitate a review of the raw data on the replicate vessels in order to more accurately evaluate the results and perform the statistical analysis. Apparently there is a statistically "significant" effect at 50 ppb which may or may not be a sampling artifact this result had a "tight" S.D. = 975. The author discounted the significane at 50 ppb but accepted a "statistically

insignificant" result at 100 ppb with a very wide S.D. = 2460. Again the author discounted the "significant" result at 200 ppb which had a "tight" S.D. = 898, but accepted the "insignificant" result at 400 ppb, which also had a "tight" S.D. = 428. The author's interpretations appear to be "one-sided" in favor of a "no effect" interpretation. EEB reserved final judgement until we review the raw data.

D. Conclusions

1. Category: ~~Invalid~~ upgrade to Core 3/26/85

2. Rationale:- needs raw data on all replicates and controls.
   - authors interpretation of the results appears "one-sided" because of discounting of significance at 50 and 200 ppb but including "insignificant" data with large S.D.'s (i.e. at 100 + 400 ppb).
   - no chemistry reported

3. Repair:
   - submit the raw data on the replicates, controls and chemistries.
   - EEB will then have to validate the statistics and decide whether to accept the study or require a new one.

SECTION C2b1

RAW DATA

WARD, G.S. 1983. Acute toxicity of MK-936 technical to embryo-larvae of eastern oysters (Crassostrea virginica).

Prepared by EG&G Bionomics, Pensacola, Florida.

Reg. No. 50658-EUP-R. EPA Accession No. 252115.

Avermectin science review

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